| Notice of Allowability | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/719,237 | YUNN-BOR ET AL. | |
| | Examiner | Art Unit | |
| | Sheridan L. Swope | 1656 | |

***-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--***

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *preliminary amendment of November 21, 2003*.

2. ☐ The allowed claim(s) is/are *11*.

3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

5. ☐ CORRECTED DRAWINGS ( as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review ( PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   **Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

1. ☒ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☒ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date 1103; 0304
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____ .
7. ☒ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

## DETAILED ACTION

Applicant's preliminary amendment of November 21, 2003, wherein Applicants cancelled Claims 1-10 and 12-14, is acknowledged. Claim 11 is pending. Claim 11, which encompasses a single invention directed to an *in vitro* method for producing 7-aminodesacetoxycephalosporanic acid (7-ADCA) using an isolated mutated penicillin expandase, is herein examined on its merits.

### *Examiner's Amendment*

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

### Specification

Replace the first paragraph of the specification with:

– This application claims the benefit of priority to U.S. Application No. 10/105,319 filed March 26, 2003, which issued as US6,699,699 on March 2, 2004, the entire disclosure of which is incorporated herein by reference in its entirety.–

On page 1, line 21, replace –deacetoxycephalosporain– with –desacetoxycephalosporin–.

### Title

Replace the title with: –*In vitro* production of 7-aminodesacetoxycephalosporanic acid using isolated mutated penicillin expandases–.

**Abstract**

On line one of the abstract, replace –activity on– with –activity, than the wild-type expandase, on–. On line three of the abstract, replace –(7-ADCA), which– with –(7-ADCA). The–.

***Allowable Subject Matter***

Claim 11 is allowed.

The following is an examiner's statement of reasons for allowance:

The elected claim, Claim 11, is limited to an *in vitro* method for producing 7-ADCA using an isolated mutated penicillin expandase, derived from a Streptomyces species wild-type expandase, wherein the variant has mutations at positions corresponding to one or more of methionine 73, glycine 79, valine 275, leucine 277, cysteine 281, and glycine 300 of the protein encoded by residues of 232-1164 of SEQ ID NO: 1. It is noted that the instant invention specifically recites an *in vitro* method using an isolated enzyme, as set forth by the Restriction/Election Requirement mailed January 8, 2003 for parent application, US10/105,319 filed March 26, 2002, which issued as US6,699,699 on March 2, 2004.

The instant invention is free of the art.

It is noted that Lee et al, 2002 (Table 2; IDS) teach a variant penicillin expandase derived from S. clavuligerus penicillin expandase, wherein the variant has a $Gly^{300}Asn$ substitution. Lee et al further teach that said variant converts penicillin G to deacetoxycephalosporin (DAOC), while a variant having $Gly^{300}Asn$ and $Tyr^{304}Phe$ substitutions can convert both penicillin N and penicillin G to DAOC. However, Lee et al is not prior art because it was not available to the public until March 27, 2002 (see enclosed evidence from the National Library of Medicine and

the Science Direct Database), while the instant invention has a valid priority date of March 26, 2002.

It is known in the art that the pathway for production of medically important cephalosporin antibiotics is penicillin N –> DAOC –> 7-ADCA and that penicillin expandase (DAOC synthetase) catalyzes the conversion of penicillin N to DAOC (Aharonovitz et al, 1992; pg 480, parg 1; Fig 1). The penicillin N –> DAOC reaction can be performed *in vitro* with isolated components (Dotzlaf et al, 1989; Fig 9). The utility of the recited method is credible based on expression of the recited variants in heterologous host cells and analysis of the conversion of penicillin N and/or penicillin G to DAOC by an enzymatic assay (Tables 1 and 2) and in light of Dotzlaf et al, wherein an *in vitro* assay using isolated components is taught. Furthermore, compared to the wild-type protein encoded by SEQ ID NO: 1, variants comprising M73T, S79E, V275I, L277K, C281Y, or G300V mutations have improved activity (Tables 1 & 2), which is an unexpected result. Therefore, the recited method has real-world utility for producing medically important cephalosporin antibiotics.

The instant invention encompasses an *in vitro* method using a genus of isolated polypeptides, derived from any wild-type penicillin expandase of any Streptomyces species, wherein the polypeptide is an active penicillin expandase having a substitution at one or more of the residues corresponding to M73, S79, V275, L277, C281Y, and G300 of the protein encoded by SEQ ID NO: 1. The scope of the said genus of polypeptide is enabled for the following reasons. Hsu et al, 2004 teach the alignment of pencillin expandases from three species of Streptomyces (Fig 3). Said alignment provides evidence that a person of ordinary skill in the art is enabled for identifying residues corresponding to M73, S79, V275, L277, C281Y, G300 of the

protein encoded by SEQ ID NO: 1. The Streptomyces clavuligerus penicillin expandase taught by Kovacevic et al, 1989 (IDS) is identical to the protein encoded by SEQ ID NO: 1 herein. A homology search using the sequence for the expandase of Kovacevic et al provides further evidence that the instant invention is enabled. Results from a NCBI BLAST search identified 16 pencillin expandases having 100-53% identity with the expandase of Kovacevic et al (see enclosed search results). A person of ordinary skill in the art would be able to identify the residues in each homologous expandase that correspond to M73, S79, V275, L277, C281Y, and G300 of the protein encoded by SEQ ID NO: 1 herein. Thus, the skilled artisan is enabled for making the recited genus of polypeptides, testing whether the polypeptides having penicillin expandase activity using standard methods in the art, and using isolated mutated polypeptides having penicillin expandase activity in a *in vitro* method for producing DAOC, which can then be used for production of 7-ADCA. For these reasons, the specification is enabling for the full scope of the recited method of producing 7-ADCA using variant penicillin expandases, derived from a Streptomyces species wild-type expandase, wherein the variant has mutations at positions corresponding to one or more of methionine 73, glycine 79, valine 275, leucine 277, cysteine 281, and glycine 300 of the protein encoded by residues of 232-1164 of SEQ ID NO: 1.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Any inquiry concerning this communication should be directed to Sheridan L. Swope whose telephone number is 571-272-0943. The examiner can normally be reached on M-F; 9:30-7 EST.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Kathleen Kerr can be reached on 571-272-0931. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on the access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Sheridan Lee Swope, Ph.D.
Art Unit 1656

SHERIDAN SWOPE, Ph.D.
PATENT EXAMINER